Thermal Inactivation of *Salmonella* on Cantaloupes Using Hot Water

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ABSTRACT: The inactivation of *Salmonella* on cantaloupes using hot water was investigated. Whole melons, inoculated with a cocktail of *Salmonella* isolates, were subjected to thermal treatments of various lengths in water at 65 °C, 75 °C, and 85 °C. Treatment with water at 85 °C for 60 and 90 s resulted in reductions of up to 4.7 log colony forming units (CFU) per square centimeter of rind. However, the rind of melons treated at 85 °C for 90 s were noticeably softer than the rind of melons treated for 60 s. Thermal penetration profiles were measured and computer simulations were conducted to verify the effect of hot water treatment conditions on the internal temperatures of cantaloupe melons. Experimental and simulation data indicated that the internal temperature of melons treated with hot water did not increase rapidly compared with the rind temperature. Regardless of the process temperature used, the temperature of the edible flesh, 10 mm from the surface of the rind, remained at least 40 °C cooler than the surface temperature of cantaloupe melons. These results demonstrate the utility of hot water for the inactivation of *Salmonella* on cantaloupes and provide a framework to producers of fresh-cut melon for the potential use of hot water as an intervention treatment.

Keywords: cantaloupe, surface pasteurization, salmonella, efficacy, decontamination

Introduction

Cantaloupe melons have been implicated in 6 outbreaks of *Salmonella* because 1990 (CDC 2002). A U.S. Food and Drug Administration (USFDA) survey conducted in response to an outbreak of *S.* Saphra in 1997 (Mohle-Boetani and others 1999) indicated that approximately 5% of imported melons were positive for *Salmonella* (USFDA 2001). Three outbreaks of *Salmonella* Poona during successive years (from April to June 2000, April to May 2001, and March to May 2002) resulted in a total of 154 reported cases. All 3 outbreaks were attributed to the consumption of melons imported from Mexico (CDC 2002). In response to these outbreaks, on October 28, 2002, the USFDA issued an import alert detaining all cantaloupes originating in Mexico offered for entry at U.S. ports (USFDA 2002). More recent surveys, however, demonstrate that melons grown in the United States are just as likely to be contaminated as those grown in Mexico (Castillo and others 2004).

A large number of sanitizing treatments for cantaloupe have been investigated in our laboratory, all with limited success. Chlorine, hydrogen peroxide, trisodium phosphate, phosphoric acid, and nisin-EDTA treatments were only partially effective in reducing microbial populations, achieving reductions of approximately 2 to 4 log colony-forming units (CFU)/cm² (Sapers and others 2001; Ukuku and others 2004; Ukuku and Fett 2004a 2004b; Annous and others 2005a). In addition, when inoculated target bacteria were allowed to reside on the melon surface for more than 24 h, the efficacy of sanitizers was reduced dramatically (Ukuku and Sapers 2001). This observation was attributed to attachment of cells to inaccessible sites within the cantaloupe netting and/or the forma-

tion of bacterial biofilms containing a protective matrix of exopolysaccharide (Annous and others 2004, 2005b).

Hot water immersion has been used previously to inactivate *Salmonella* on the surface of cantaloupes (Annous and others 2004; Ukuku and others 2004). Significant reductions (in excess of 5 logs) were achieved when cantaloupes were treated at 76 °C for 3 min using commercial-scale equipment (Annous and others 2004). Ukuku and others (2004) achieved a 3-log reduction with a treatment of 60 s at 97 °C using a laboratory-scale water bath. However, these 2 investigations simply demonstrated the potential value of using hot water immersion as a method of inactivating bacteria on the melon rind. While temperature penetration profiles for melons treated at different temperatures indicated that cantaloupe surface and subsurface (5.1 mm) temperatures, but not flesh temperature (10 mm), increased rapidly (Annous and others 2004). A detailed analysis of heat transfer was not performed.

Therefore, the main goal of this research was to perform numerical analyses of heat transfer to melon surfaces and internal portions to develop a simulation model for temperature penetration. The secondary objective was to evaluate the efficacy of experimental temperatures used in reducing *Salmonella* populations inoculated on the melon rind.

Materials and Methods

Bacteria

All isolates used in this study were from the USDA-ARS-ERRC (Wyndmoor, Pa., U.S.A.) culture collection. Stock cultures of *Salmonella* Poona RM2350 = California Dept. of Health Services 00A3563 (2000 cantaloupe outbreak), *S.* Poona G91-1574 (1991 cantaloupe outbreak), *S.* Michigan (human feces, linked to cantaloupe consumption), *S.* Oranienburg 389 (isolated from cantaloupe grown in Texas), and *S.* Saint Paul 02-517-1 (isolated from cantaloupe grown in Mexico) were stored in tryptic soy broth (TSB; BBL/Difco, Sparks, Md., U.S.A.) containing 30% glycerol at

–80 °C. Working cultures were maintained on tryptic soy agar (TSA; BBL/Difco) plates at 4 °C.

Cantaloupes, surface area calculation, and inoculation procedures

Cantaloupes (*Cucumis melo* L.; weighing between 1026 and 1638 g) were obtained from local supermarkets and stored at 4 °C for no more than 2 d before use. Before inoculation, the surface area of the rind of each melon was calculated as previously described (Annous and others 2005a). Inoculum preparation was a modification of previously described protocols (Annous and others 2004). A loop full of each culture from a TSA plate was transferred into 10 mL of TSB and was allowed to grow at 35 °C for approximately 8 h. This culture was used to inoculate 500 mL of fresh TSB at 0.01% (v/v), which was then incubated overnight at 35 °C. Cell population of each culture was determined to be 9.3 to 9.7 $\log_{10}$ CFU/mL by surface plating onto TSA. Cultures were centrifuged (6740 × *g*) for 10 min, washed once with 200 mL sterile deionized water (SDW), centrifuged again, and then re-suspended in 200 mL of SDW. Culture suspensions of all 5 strains were combined and added to 3 L of SDW to give a final volume of 4 L. Cocktail populations were determined by surface plating onto TSA to be approximately 9 $\log_{10}$ CFU/mL. Cantaloupes were inoculated by total submersion into the inoculum for 5 min without agitation and then removed, drained, and dried for 1 h in a laminar flow hood. Melons were stored in plastic tubs at 4 °C for 24 h before hot water treatment.

Hot water treatments

Thermal treatments were conducted in a specially fabricated treatment vessel. The treatment vessel was constructed using a 75-L stainless-steel cylindrical canister (McMaster Carr, Dayton, N.J., U.S.A.) wrapped with 13-mm closed-cell foam insulation to minimize heat loss. Water in the vessel was heated and maintained at the desired temperature with a 3000-watt electric immersion heater (Cleveland Process Corp., Homestead, Fla., U.S.A.), controlled with a custom built temperature controller. A centrifugal pump (Little Giant Pump Co., Oklahoma City, Okla., U.S.A.) with a discharge of 14 L/min provided gentle mixing within the vessel but without direct impingement on the submerged cantaloupes. Temperature was monitored using a class "A" platinum resistance temperature device and the system was set such that the vessel maintained temperatures within ±0.5 °C, even upon addition of single test cantaloupes into the tank (temperatures were recorded every 2 s). Inoculated cantaloupes were removed from refrigerated storage and submerged in water held at 65 °C, 75 °C, or 85 °C for various lengths of time. Control cantaloupes were inoculated as above and treated in water at room temperature (RT = 20 °C) under the same conditions to ensure that observed reductions were a result of thermal treatment as opposed to simple washing. Following treatment, melons were removed and placed into ice water until microbiological analysis was performed (15 to 45 min). Experiments were performed in triplicate and repeated twice.

Microbiological analysis

Surviving populations of *Salmonella* were recovered as described previously (Annous and others 2004). Briefly, the entire cantaloupe rind was removed using a fruit peeler (Muro Peel-All Fruit Peeler, Muro Corp., Tokyo, Japan), placed into a sterile blender jar, weighed, combined with 4 equivalent volumes of sterile 0.1% peptone water (PW), and blended at medium speed for 1 min in a commercial blender (Waring Products, Torrington, Conn., U.S.A.). Homogenates were filtered through a stomacher bag (Spiral Biotech, Bethesda, Md., U.S.A.), and equal volumes were dispensed into sterile 10-mL tubes. Filtrates were diluted as required, plated onto TSA, and incubated at 35 °C for approximately 2 h to allow for recovery of injured cells. Plates were then overlaid with xylose lysine tergitol-4 agar (XLT-4; BBL/Difco), allowed to dry, and then incubated overnight at 35 °C. Resultant colonies were enumerated, and survivors were expressed as $\log_{10}$ CFU/cm² of cantaloupe rind.

Heat transfer during surface pasteurization

After the temperature of the water in the treatment vessel was stabilized to a set-point, a cantaloupe was immediately immersed into the water. Because of the temperature differential between the hot water and the cold melon, heat transfer occurred. Thermal energy was 1st transferred from the hot water to the surface of the cantaloupe and into the interior of the cantaloupe along the radial direction. The cantaloupe was considered a spherical shell (Figure 1). In a spherical geometry, the transfer of thermal energy from hot water to the cantaloupe is governed by a 1-D unsteady-state heat conduction equation (Incropera and DeWitt 1996) and can be expressed as follows:

$$\rho C_P \frac{\partial T}{\partial t} = k \left(\frac{\partial^2 T}{\partial r^2} + \frac{2}{r} \frac{\partial T}{\partial r} \right) \tag{1}$$

This equation has 2 boundary conditions. On the outer surface directly exposed to hot water, that is, at $r = R_1$, the boundary condition is

$$\frac{\partial T}{\partial r} = -\frac{h}{k}(T - T_w) \tag{2}$$

The central cavity of the cantaloupe was considered hollow because the majority of the space in the core is filled with air. For the short treatment time used in hot water pasteurization, it was assumed that no thermal energy was transferred to the core of the cantaloupe. Therefore, the inner surface was treated as an insulated area. On the inner surface, that is, at $r = R_2$, the boundary condition can be expressed as follows:
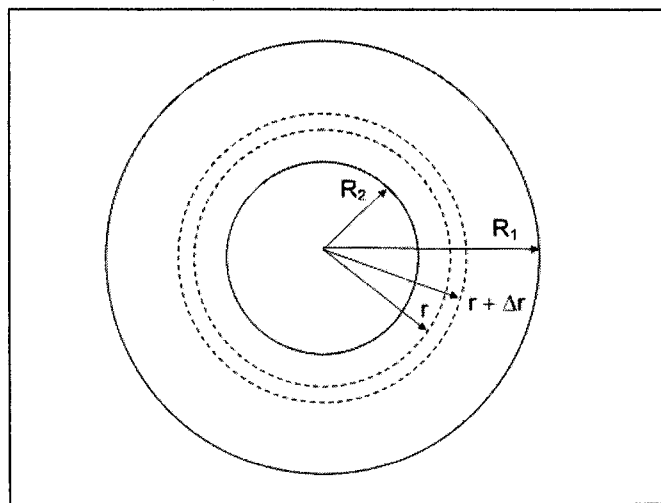
$$\frac{\partial T}{\partial r} = 0 \tag{3}$$

Figure 1—Schematic representing spherical shell geometry

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For Eq. 1 to 3, T is the temperature (°C) at any given time and location between R_1 and R_2 of a cantaloupe, T_w is the hot water temperature (°C), t is the heating time (s) after the cantaloupe is immersed into the treatment vessel, r is the radius (m), h is the overall heat transfer coefficient (W/m²°C), k is the thermal conductivity (W/m°C), ρ is density (kg/m³), and C_p is the specific heat of the cantaloupe (J/kg°C). For the initial conditions of Eq. 1, it was assumed that the temperature was uniform throughout the cantaloupe.

Reference temperature measurement

To obtain the temperature history at the reference location used in numerical analysis, a specially fabricated temperature probe was used. The temperature probe was constructed using a 40-gauge type "T" thermocouple wire inserted into a 0.5-inch-long (1.27 cm) 26-gauge hypodermic needle. The welded sensing tip of the thermocouple was fitted exactly into the opening at the sharp end of the hypodermic needle. High-temperature superglue was applied to secure the thermocouple tip to the needle opening. When inserted into the sample, the sensing tip of the thermocouple was exactly 1 cm below the cantaloupe surface. During experiments, this temperature probe was inserted into randomly selected locations. Temperature histories collected from these locations were used as reference temperatures in the numerical analysis described subsequently. Two additional 30-gauge thermocouples were used to gather reference temperatures. One was used to measure the temperature of the hot water in the processing vessel. The exact time when heating started was determined with this thermocouple. The other thermocouple was inserted into the cork layer of the melon rind to a depth of 1 mm below the surface. This thermocouple was labeled the surface probe. Temperature profiles were collected using the data acquisition system previously described by Annous and others (2004).

During measurement of the reference temperature, the hot water in the vessel was at 65 °C. The temperatures of the hot water, melon surface, and at 1 cm below the surface were recorded at 2-s intervals. Experiments were performed in triplicate and the overall heating time varied between 5 to 10 min.

Numerical analysis of heat conduction

The objective of numerical analysis was to simulate and understand the temperature distribution in cantaloupes subjected to surface pasteurization by hot water immersion. The one-dimensional heat conduction equation (Eq. 1) governing the surface pasteurization process was solved using a computer program developed by Huang (2004). The program was originally developed to solve 1-D unsteady-state heat conduction in a cylindrical coordinate system but was modified to the spherical coordinate system. The numerical algorithm in the computer program was based on an explicit scheme of the finite difference method (Chandra and Singh 1995). To guarantee convergence during numerical analysis, the time step (Δt) and the space step (Δr) were carefully chosen such that

$$\Delta t \leq \frac{\Delta r^2 \rho C_p}{2k}.$$ (4)

During numerical analysis of heat conduction in the cantaloupes, it was assumed that the plant tissue between R_1 and R_2 was homogeneous. Temperature penetration data collected at same depth for different location of the same melon similar (data not shown), suggesting uniformity among the tissues between R1 and R2. It was also assumed that the physical properties of cantaloupe were relatively stable throughout the process of hot water surface

pasteurization, and were therefore treated as constants. The thermal conductivity (k = 0.571 W/m°C) and density (ρ = 930 kg/m³) of cantaloupe were taken from Rahman (1996). The specific heat (C_p = 3969 J/kg°C) of cantaloupe was taken from Lutz and Hardenburg (1968). The average diameter of cantaloupe tested was 14 cm, and the thickness of the flesh was approximately 3.6 cm.

To solve the differential equation used to describe the heat transfer during surface pasteurization of cantaloupes, the surface heat transfer coefficient (h) was unknown but needed in the boundary condition expressed in Eq. 2. To solve the differential equation (Eq. 1), a numerical algorithm was developed to simultaneously determine the surface heat transfer coefficient and the temperature distribution within the cantaloupe. To achieve this goal, the numerical iteration was initiated with an arbitrary small value of h. After each iteration cycle, a temperature history at a certain reference location was generated. This temperature history was compared with the true temperature measured at the same location. The least-squares method was used to determine the difference between the computer-generated and the real-time temperature histories at the same location. A pseudo-R^2 value was computed (Eq. 5). In this equation, T_{ci} and T_{mi} represent the computer-generated temperature and the measured temperature at the time point of t_i, respectively. T_{avg} is the average of the computer-generated temperature at this reference location, and n is the total number of temperature points.

$$R^2 = 1 - \frac{\sum_1^n \left(T_{ci} - T_{mi}\right)^2}{\sum_1^n \left(T_{ci} - T_{avg}\right)}$$ (5)

After each iteration, a small increment of h was used to restart the iteration process. The iteration would continue until a termination criterion was met. In this study, the termination criterion was set as $R^2 \geq 0.99$.

Results and Discussion

Thermal inactivation of *Salmonella* on cantaloupe surfaces

Inoculation, storage, and processing conditions of melons used in this study were similar to those used in previous work (Annous and others 2004). Inoculated melons were held overnight at 4 °C to allow for strong bacterial attachment and/or biofilm formation before treatment. Ukuku and Sapers (2001) demonstrated that washing of melons using chlorine or peroxide within a short time after inoculation resulted in higher reductions than when melons were refrigerated for 24 h or more before washing. They speculated that strong attachment or the formation of microbial biofilms was responsible for the lack of sanitizer efficacy. We have shown recently that biofilm formation begins within 2 h following the introduction of *Salmonella* onto the cantaloupe surface (Annous and others 2004, 2005b). Cells within a biofilm are encased in a protective layer of polymer that may protect them from inactivation by sanitizer treatments. We used the conditions described so that we could test our thermal treatments against strongly attached cells, or those encased within a biofilm, making them more resistant to inactivation.

The laboratory-scale work reported here was performed in a 75-L vessel (designed and fabricated at ERRC) and was not subject to the mass/solution volume difficulties encountered in our previous study (Annous and others 2004). Our previous laboratory-scale

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Table 1—Reductions in *Salmonella* inoculated onto whole cantaloupes following hot water treatment[a,b]

Treatment time (s)	Water temperature (°C)			
	Room temperature	65	75	85
10	0.07±0.20	0.52±0.26	1.20±0.56	1.80±0.44
30	−0.25±0.16	0.82±0.31	1.46±0.47	3.59±0.49
60	−0.15±0.18	1.19±0.38	2.09±0.28	4.68±0.35
90	−0.46±0.01	0.96±0.70	3.31±0.89	4.46±1.05

[a]Population reduction ($\log_{10}$ colony-forming units [CFU]/cm^2) based on control populations of 5.37 ± 0.41.
[b]Values represent means ± standard deviation from 6 melons.

work submerged test cantaloupes in 4 L of water in bench-top water baths. The addition of a 1200 to 1500 g cantaloupe to 4 L of hot water caused an initial drop of approximately 6 °C. Because of this decrease in initial temperature, we had to increase the treatment time of cantaloupes from 3 to 6 min. The size of the tank and volume of water used in the present study precluded any such temperature loss in our current work.

Reductions in levels of *Salmonella* achieved using the treatment vessel described previously further underscore the utility of hot water as a method for decontaminating the surface of cantaloupes (Table 1). Water at 85 °C was by far the most effective treatment in

reducing populations of *Salmonella*, with heating times of both 60 and 90 s resulting in approximately 4.5 $\log_{10}$ CFU/cm^2 reductions. We observed that the rind of melons treated for 90 s at 85 °C were noticeably softer than the rind of control melons suggesting heat damage to the surface of the rind; however, treatment for 60 s at 85 °C resulted in similar reductions in populations of *Salmonella*, but without the loss in firmness of the rind (Annous and others 2004). Therefore, at 85 °C, a heating time of 60 s would be preferred.

Treatments at 65 °C or 75 °C resulted in correspondingly lower reductions in levels of *Salmonella* (Table 1). Ten seconds at 65 °C resulted in an average reduction of only 0.5 $\log_{10}$ CFU/cm^2. Water at 65 °C was not able to effect more than a 1 log reduction in *Salmonella* populations, even after 90 s, and therefore would not be a recommended process temperature. Treatments at 75 °C were far more effective than those at 65 °C, with a treatment time of 90 s resulting in a reduction of 3.31 $\log_{10}$ CFU/cm^2.

Reductions in *Salmonella* populations on the surface of artificially inoculated cantaloupes using hot water immersion at 80 °C for 90 s (Table 1) or 76 °C for 3 min (Annous and others 2004) were 4.5 and 5.3 logs, respectively, compared with a maximum of 3 log reductions obtained with aqueous sanitizers and various other physical treatments (Ukuku and Sapers 2001; Ukuku and Fett 2004a, 2004b; Annous and others 2005a). The increased efficacy of the hot water treatment may be the result of heat transfer to bacteria in the netting and/or biofilm (Annous and others 2004, 2005a). Thus, the use of hot water immersion as a method for decontaminating the surface of cantaloupes is more effective than aqueous sanitizers and various other physical treatments tested to date. Also, when plotting the current thermal inactivation data and including the 5.3 log reduction previously achieved by heating for 3 min at 76 °C (Annous and others 2004), the regression coefficient was $R^2 = 0.99$. This would suggest that the laboratory-scale studies could be extrapolated to the pilot-scale treatment tank.

Numerical analysis of heat transfer

Figure 2 shows the comparison of the temperature histories on the surface of cantaloupe and at the reference location as measured by the thermocouples and generated by numerical analysis. Using the numerical algorithm with a $R^2 \geq 0.99$ as a termination criterion, the simulated temperature history at the reference location matched well with the temperature history at the same location measured experimentally.
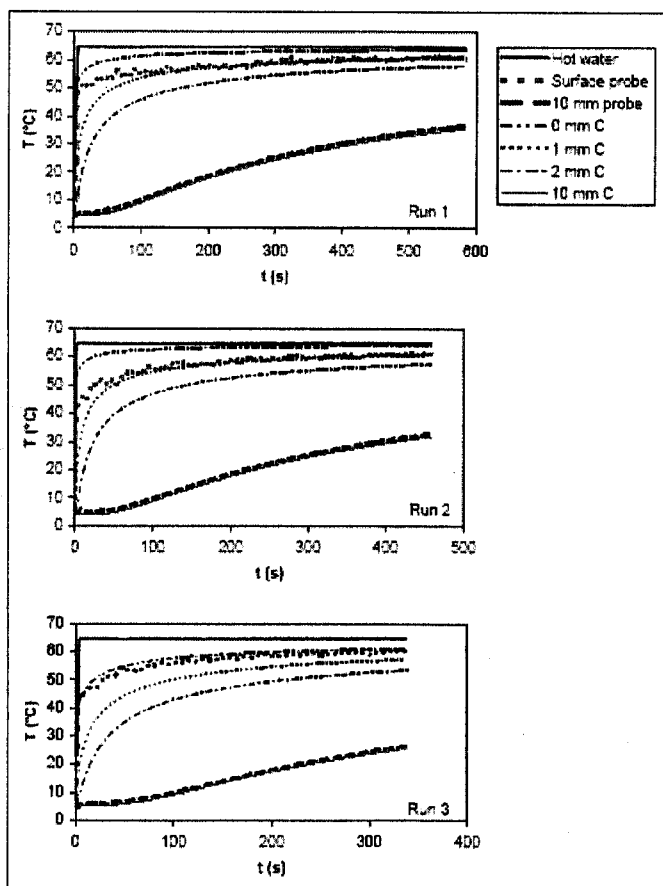
Figure 2—Experimental and simulated heat penetration curves of cantaloupe ("C" denotes simulated temperature). Thermocouples were inserted at 0 to 10 mm from the rind outer surface of the cantaloupe before submersion in 75 L of water at 65 °C for 10 min.
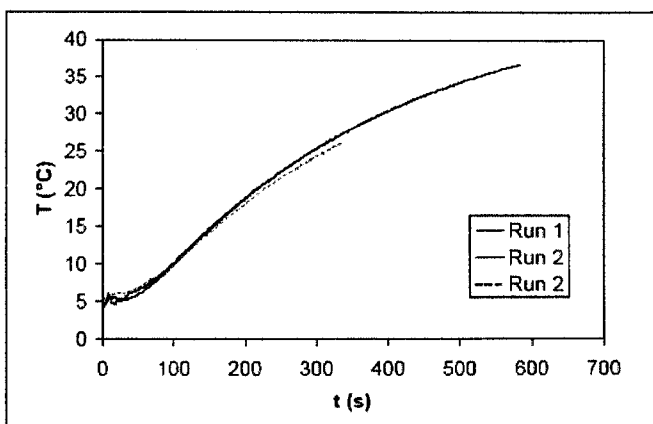
Figure 3—Experimental temperature profiles measured at 10 mm below the rind surface of cantaloupes exposed to water at 65 °C for 10 min. Each run represents 1 melon.

The surface heat transfer coefficients (h) estimated from the numerical analysis were 573, 1190, and 1740 W/m²°C for 65 °C, 75 °C, and 85 °C temperature profile curves obtained from experiments, respectively. Although the range of the estimated values is fairly wide, the temperatures measured at the reference location (1 cm below the surface) do not differ significantly from each other (Figure 3). This observation can be explained by the fact that there was little resistance to heat transfer between the hot water and the surface of cantaloupes. The conduction of heat from the surface to the interior of cantaloupes was the rate-limiting factor during pasteurization of cantaloupes. This explanation is supported by the fairly large Biot number (Eq. 6).

$$Bi = \frac{hR_1}{3k}$$

(6)

The calculated *Bi* number for the 3 tests was 46, 97, and 142, respectively. These values are significantly greater than 1 (*Bi* >> 1), indicating that conduction was the dominant source of heat resistance during heat transfer (Incropera and DeWitt 1996). Therefore, the choice of h does not affect the simulation results of the temperature profiles below the surface of cantaloupes. For the sake of convenience, the average (1168 W/m²°C) of the 3 h values estimated from numerical analysis was used in the computer simulation of temperature distribution below the surface of the cantaloupes.

Figure 2 also shows the simulated temperature histories on the surface and at locations 1 and 2 mm below the surface of the melons. It is worth noting that although the temperatures on the surface of cantaloupes rose rapidly, the temperatures at locations 1 or 2 mm below the surface did not respond as quickly. Because the surface probe was inserted at points slightly below the cork layer, the temperature measured by this probe does not represent the surface temperature of cantaloupes. Because the location of the surface probe was different each time the probe was inserted into

the cantaloupes, the temperature history may be slightly different between runs. Depending on the insertion depth of the surface probe, it measured the temperature at a point between the surface and 1 mm below the surface. Also, the temperature measured by the surface probe rose almost instantaneously with the surface temperature and then began to level off. This observation is likely due to the fact that the majority of the surface probe was exposed to hot water, and only a small tip of the thermocouple was inserted into the cork layer where heat was conducted through the wires of the thermocouple to the sensing tip until equilibrium in heat transfer was established between hot water and the cork layer. After equilibrium was established, the temperature sensed by this probe began to level off.

Computer simulation of temperature distribution

After the numerical algorithm was validated and the surface heat transfer coefficient determined, temperature distributions beneath the surface of cantaloupes during surface pasteurization were simulated using the computer program (Huang 2004). Figure 4 shows the temperature distribution beneath the surface of cantaloupes exposed to 75 °C and 85 °C water for 10 min. The temper-
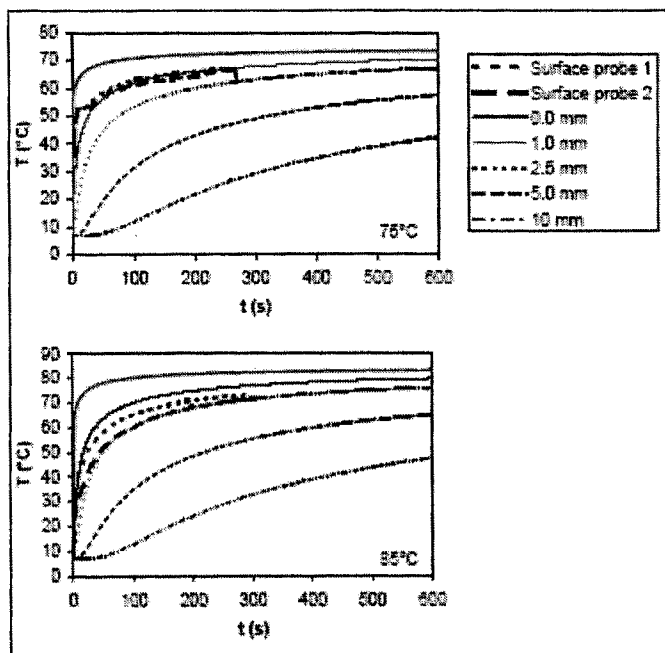

Figure 4—Simulated temperature profiles measured at 1, 5, and 10 mm beneath the rind surface of cantaloupes exposed to water at 75 °C and 85 °C for 10 min
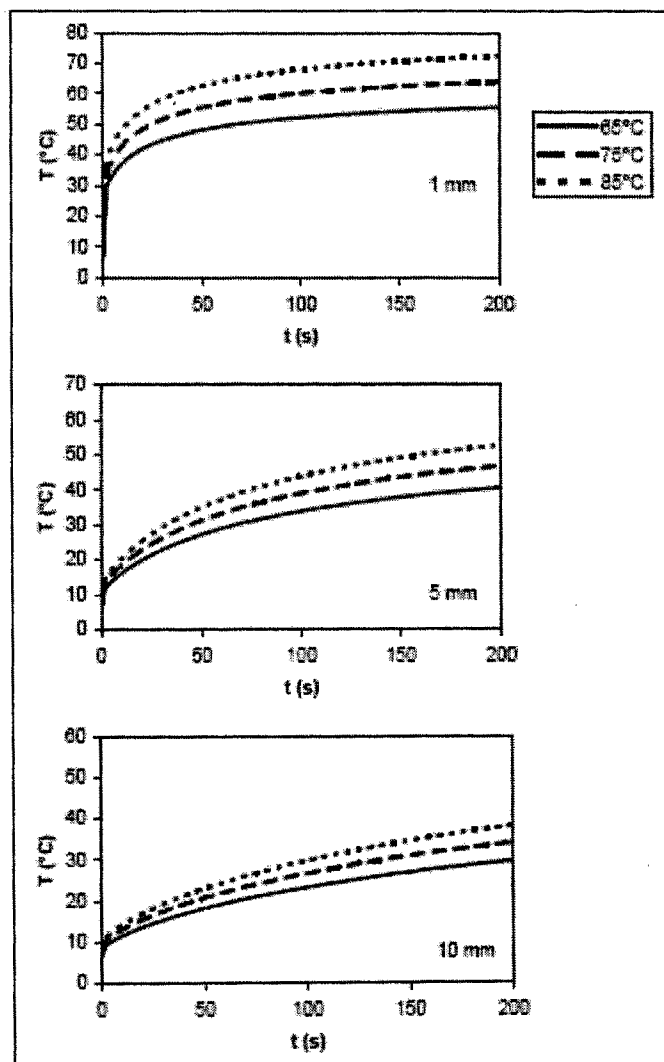

Figure 5—Simulated average temperatures at 1, 5, and 10 mm below the rind surface of cantaloupes during heating in water at 65 °C, 75 °C, and 85 °C

ature measured by surface probe 1 and 2 represents the temperatures at locations somewhere between the surface and the cork layer. Using process times of less than 100 s, the temperatures at deeper locations (5 or 10 mm below the surface) remained below 15 °C, indicating that the thermal process might not negatively affect the quality of fresh-cut cantaloupes.

To better understand energy absorption by the cantaloupe tissue, the average temperature at different depths was calculated and presented in Figure 5. Clearly the average temperature at the layer 1 mm below the surface can rise rapidly to a point lethal to *Salmonella*, while the temperatures at the layers 5 and 10 mm below the surface remain relatively low. The edible cantaloupe flesh begins at approximately 5 mm below the rind surface, indicating that surface pasteurization would enable destruction of the pathogen while allowing the flesh of the melon to remain cool.

Conclusions

The use of hot water as a method to decontaminate cantaloupe is more effective than various other washing and physical treatments tested to date. The work presented in this article demonstrates the utility of surface pasteurization to greatly reduce levels of *Salmonella* from the surfaces of cantaloupes. In addition, heat penetration analysis coupled with computer simulation of heat transfer indicates that the edible portions of cantaloupes remain cool while the temperature of the rind outer surface elevates rapidly. This is an added benefit to the use of hot water surface pasteurization. Experiments are currently under way to examine the quality and shelf life of melons exposed to various thermal treatments.

Acknowledgments

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